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Elevated serum homocysteine levels and increased risk of invasive cervical cancer in US women

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Abstract

Objectives: To explore the relationship between serum homocysteine, a sensitive biomarker for folate inadequacy and problems in one-carbon metabolism, and invasive cervical cancer.

Methods: A large case–control study was conducted in five US areas with up to two community controls, obtained by random-digit dialing, individually matched to each case. Cervical cancer risk factors were assessed through at-home interview. Blood was drawn at least 6 months after completion of cancer treatment from 51% and 68% of interviewed cases and controls. Serum homocysteine was measured by high-performance liquid chromatography, and exposure to human papillomavirus (HPV) type 16, the most prevalent oncogenic type, was assessed using an enzyme-linked immunosorbent assay. Cases with advanced cancer and/or receiving chemotherapy were excluded, leaving 183 cases and 540 controls.

Results: Invasive cervical cancer risk was substantially elevated for women in the upper three homocysteine quartiles (>6.31 μmol/L); multivariate-adjusted odds ratios ranged from 2.4 to 3.2 (all 95% CIs excluded 1.0). A trend was apparent and significant ($p=0.01$). When cases were compared with HPV-16 seropositive controls only, odds ratios were comparable.

Conclusions: Serum homocysteine was strongly and significantly predictive of invasive cervical cancer risk. This association could reflect folate, B_{12} and/or B_6 inadequacy, or genetic polymorphisms affecting one-carbon metabolism.

Introduction

For the past 25 years there has been credible speculation that folate inadequacy might be a risk factor for cervical neoplasia [1, 2]. However, observational studies of both preinvasive and invasive cervical neoplasia have produced conflicting results [3–14] and treatment trials of dysplasia with folate supplements have had mixed success [2, 15, 16].

Serum homocysteine is a sensitive indicator of folate status [17] and an emerging biomarker of folate inadequacy, as well as other problems in one-carbon metabolism [17–19]. Folate is essential for one-carbon metabolism, which encompasses amino acid metabolism, purine and pyrimidine synthesis, and formation of *S*-adenosylmethionine, the agent primarily responsible for methylation of DNA [20]. Disruption of one-carbon metabolism can interfere with DNA synthesis, repair,

and methylation and thus promote carcinogenesis [21]. In addition, chromosomal fragile sites, which can be caused by folate inadequacy, may also be involved in cancer [22]. The human papillomavirus (HPV), the major causal agent of cervical cancer [23, 24], may integrate into the host genome at or near fragile sites [25–27], providing another mechanism whereby folate and one-carbon metabolism may influence cervical carcinogenesis.

Efficient one-carbon metabolism requires not only folate, but also adequate levels of several other vitamins and optimal activity of several enzymes. In one-carbon metabolism, homocysteine accepts a one-carbon group from folate to form methionine in a vitamin B_{12}-requiring reaction or, alternatively, homocysteine is degraded in a vitamin B_6-requiring reaction [28]. Impairment of either pathway may result in the accumulation of homocysteine [28]. In human populations, elevated levels of homocysteine have been associated with low levels of folate, vitamin B_{12}, and vitamin B_6 [19, 29, 30], and with a common polymorphism ($^{667}C \rightarrow T$) in the methylenetetrahydrofolate reductase (MTHFR) gene, which reduces enzyme activity [18]. Thus, serum homocysteine can be a sensitive, integratory biomarker of disruption in one-carbon metabolism [17–19, 28].

Because of continued interest in diet and cervical neoplasia, and in order to evaluate directly the importance of efficient one-carbon metabolism in the etiology of the disease, we examined the association between serum homocysteine levels and risk of invasive cervical cancer in a large, multicenter, community-based case-control study in US women.

Methods

Study design

Eligible subjects were all incident cases of histologically confirmed, primary invasive cervical cancer, aged 20–74 years, identified between April 1982 and January 1984 in five US areas reporting to the Comprehensive Cancer Patient Data System. The study sites were centered around Birmingham, AL; Chicago, IL; Denver, CO; Miami, FL; and Philadelphia, PA. Up to two potential controls, matched on age (±5 years), ethnicity (white, black, Hispanic), and neighborhood (first six digits of a 10-digit telephone exchange), were identified by random-digit dialing for each case. Trained staff conducted interviews in the subjects' homes with structured questionnaires to obtain detailed information on demographic characteristics, sexual behavior, reproductive and menstrual history, exogenous hormone use, personal and familial medical history, smoking, and diet. The methodology for the overall study has been described [31, 32].

Blood samples were drawn at least 6 months after completion of any treatment for cervical cancer (days after treatment: 10th, 50th, 90th percentiles = 190, 342, 669 days, respectively). Treatment included surgery (44%), radiation (18%), or both (28%). A small percentage of subjects (4%) also received chemotherapy (6% of subjects were missing treatment information). The blood was allowed to clot at room temperature for 40–60 minutes before being centrifuged, and serum samples were stored at −70 °C.

All study participants provided informed written consent prior to study initiation. The study was approved by the Institutional Review Boards of the National Cancer Institute and the five participating study centers.

Participation rates

A total of 1281 women were interviewed (480 of 658 eligible cases and 801 of 1114 eligible controls). Blood was obtained from 245 cases and 545 controls (51% and 68% of those interviewed, respectively). Reasons for non-participation in the blood draw included death (17% of cases, 0.4% of controls), contact and scheduling difficulties (15%, 17%), subject refusal (9%, 13%), hospital refusal (6%, 0%), cases who were not yet 6 months post-treatment at the completion of the study (2%, 0%), and unsuccessful blood draws (2%, 1%).

Cases who received chemotherapy treatment (n = 11) and/or who had advanced (stage III or IV) cervical cancer (n = 17) were excluded from the epidemiologic analyses to minimize the possibility that advanced disease or poor health would influence the results. Also excluded were cases with non-squamous cell cervical cancer (n = 28), and women whose ethnicity was other than white, black, or Hispanic (seven cases, two controls). Three cases and one control had insufficient serum for the homocysteine assay, and serologic HPV data were unavailable for one case and one control. These exclusions were not mutually exclusive. Included in the epidemiologic analyses were 183 cases and 540 controls.

Laboratory methods

Homocysteine. Serum homocysteine analyses were conducted using a modification of a high-performance liquid chromatography method [33]. Cases and their matched controls were assayed consecutively within the

same batches. Eight blinded quality control samples (two aliquots from each of four donors at low, intermediate, and high homocysteine levels) were included in each batch of 80 samples. These quality control samples were monitored by the NCI collaborators based on rules by Westgard *et al.* [34], and four unacceptable batches were repeated. The coefficient of variation was 12%, based on repeated measurements of the blinded quality control material over 6 months of assays. This included both within- and between-batch variability, and was calculated using the variance component estimation procedure in SAS [35].

HPV type 16. The test for HPV type 16 serum antibodies used a well-characterized HPV-16 virus-like particle, enzyme-linked immunosorbent assay (ELISA) [36]. Samples were tested in duplicate and, prior to being averaged, the optical density (OD) readings of each duplicate were adjusted according to results of three control samples run in triplicate in each batch, to control for between-day and between-batch variability. An OD < 0.904 was classified as seronegative; an OD > 1.017 was classified as seropositive; and ODs between these values (3.6% of subjects tested) were considered indeterminate [37].

Statistical analysis

The odds ratio (OR) was the measure of association used to estimate the relative risk of cervical cancer. Homocysteine quartiles were based on the frequency distribution among the controls. Logistic regression was used to obtain maximum-likelihood estimates of the OR and a 95% confidence interval (CI), while adjusting for potential confounders [38]. Comparable ORs were found using conditional and unconditional regression models, adjusted for age, ethnicity, and study site. Therefore, unconditional regression models which retained all the cases and controls whose matched pairs did not participate in the blood draw portion of this study, are presented. Control for confounding was considered adequate when the addition of a potential confounder or an increase in the number of strata of a confounder did not change the adjusted OR by 0.1 or more. Unless otherwise specified, ORs in the text and tables are adjusted for study design factors (age, ethnicity, study site) and exposures related to risk in this study, specifically, HPV-16 serologic status, number of sexual partners, age at first intercourse, years since last Papanicolaou (Pap) smear, number of pregnancies, smoking status and intensity, oral contraceptive use, education, and income. Age at first intercourse and number of sexual partners were included, along with HPV-16 serologic status, to give a more complete picture of the history of HPV infection. Smoking status and intensity was a composite variable with the following categories: non-smoker, former smoker of <20 cigarettes/day, former smoker of ≥20 cigarettes/day, current smoker of <20 cigarettes/day, and current smoker of ≥20 cigarettes/day. Tests for trend were obtained by assigning to each homocysteine quartile the median level of the controls and treating this as a continuous variable. Effect modification was assessed by adding interaction terms to the multivariate-adjusted model. All statistical tests were two-tailed.

In order to examine whether systemic effects of cancer or treatment for cancer affected blood homocysteine levels, mean homocysteine levels of cases were examined by stage of cancer. In addition, mean levels in Stage I and II cases were examined by treatment. Log-transformed means were adjusted using analysis of covariance for factors that could influence blood homocysteine levels, disease progression, and/or chosen treatment, either directly or indirectly.

Results

The distribution of cases was not significantly different from that of the controls on age, ethnicity, or study site (Table 1). Potential controls had been individually matched to eligible cases on these factors in the original study design.

Women with homocysteine levels in each of the three highest quartiles had statistically significantly elevated risks of invasive cervical cancer (OR = 2.4–3.2 in the multivariate, HPV-adjusted model, p for trend = 0.01) relative to women in the lowest quartile (Table 2). ORs were not substantially altered by addition of HPV-16 serologic status or other cervical cancer risk factors to the model, suggesting that there was little uncontrolled confounding. These 2–3-fold increased risks were observed at serum homocysteine levels ≥6.31 μmol/L. This level is well within the US range for women reported in the Third National Health and Nutrition Examination Survey (5th–95th percentiles = 3.7–10.4, 4.1–10.2, and 4.9–11.6 μmol/L for women aged 20–39, 40–59, and ≥60 years, respectively) [39].

We compared models with and without education and income, indicators of socioeconomic status. The inclusion of these two variables slightly attenuated the ORs (from 3.2 to 2.9 in the highest quartile) and were therefore included in the models to provide conservative estimates of risk. The addition of vitamin C or carotenoid intake to the model modestly increased, rather than decreased, the homocysteine OR (data not shown).

Table 1. Distribution of cases and controls by demographic characteristics matched in the original study design

	Cases (n = 183)		Controls (n = 540)		Chi-square *p*-value cases *vs.* controls
	No.	Percentage	No.	Percentage	
Age (years)					
<35	43	23	136	25	
35–44	51	28	161	30	
45–54	40	22	114	21	
55+	49	27	129	24	0.84
Ethnicity					
White	121	66	365	68	
Black	44	24	137	25	
Hispanic	18	10	38	7	0.47
Study site					
Birmingham	41	22	104	19	
Chicago	31	17	120	22	
Denver	51	28	132	24	
Miami	25	14	73	14	
Philadelphia	35	19	111	21	0.51

Table 2. Adjusted odds ratios of invasive cervical cancer by serum homocysteine levels

Serum homocysteine quartile	Range of homocysteine levels (μmol/L)	No. of cases (n = 183)	No. of controls (n = 540)	Study design – adjusted OR[a] (95% CI)	Multivariate-adjusted (without HPV status) OR[b] (95% CI)	Multivariate-adjusted (with HPV status) OR[c] (95% CI)
Quartile 1	<6.31	20	134	1.0	1.0	1.0
Quartile 2	6.31–8.07	44	135	2.20 (1.2–4.0)	2.07 (1.1–4.0)	2.40 (1.2–4.8)
Quartile 3	8.08–10.54	60	135	3.04 (1.7–5.5)	2.78 (1.5–5.3)	3.22 (1.7–6.4)
Quartile 4	>10.54	59	136	2.97 (1.7–5.4)	2.59 (1.4–5.0)	2.91 (1.5–5.9)
				p_{trend} = 0.001	p_{trend} = 0.014	p_{trend} = 0.011

[a] Adjusted for age, ethnicity, and study site.

[b] Also adjusted for number of sexual partners, age at first intercourse, years since last Pap smear, number of pregnancies, smoking status and intensity, oral contraceptive use, education, and income.

[c] Adjusted for HPV-16 seropositivity and all factors in notes a and b.

No statistically significant effect modification was found between serum homocysteine and oral contraceptive use, age at first intercourse, or number of sexual partners.

HPV is believed to be responsible for more than 90% of all invasive cervical cancers [23, 24]. After completion of treatment, 36% (n = 77) of the 214 squamous cervical cancer cases tested seropositive for HPV-16, the most common oncogenic HPV type, while only 15% (n = 79) of controls tested seropositive. Fourteen cases and 15 controls had indeterminate HPV-16 status. The risk for invasive cervical cancer with a seropositive HPV-16 test was 4.60 (95% CI 2.9–7.3) in a multivariate-adjusted model excluding indicators of HPV infection (age at first intercourse, number of sexual partners).

We examined the association between homocysteine and cervical cancer risk using only controls seropositive for HPV-16. All cases were used in this analysis because we assumed all cases had been exposed to oncogenic HPV at one time. High homocysteine levels were clearly associated with increased cervical cancer risk after controlling for exposure to oncogenic HPV in this manner (Table 3). In addition, comparable results were observed when only HPV-16 seropositive cases were included; adjusted ORs for the lowest to highest homocysteine quartiles were 1.0, 1.4, 3.2, and 1.7. In both these analyses, smaller numbers of subjects led to less stable estimates of risk and broader confidence limits.

We also assessed the relationship between homocysteine levels and infection with oncogenic HPV. Among the controls, homocysteine levels were not predictive of detection of HPV-16 antibodies. Adjusted ORs for the lowest to highest homocysteine quartiles were 1.0, 1.0, 1.0, and 1.3.

Subjects who participated in the blood draw component of the study, relative to all those who participated

Table 3. Adjusted odds ratios of invasive cervical cancer by serum homocysteine levels in women with a history of HPV infection

Serum homocysteine quartile	Range of homocysteine levels (μmol/L)	No. of cases[a] (n = 183)	No. of HPV-16 seropositive controls (n = 79)	All cases *vs.* HPV-16 seropositive controls OR[b] (95% CI)
Quartile 1	<6.31	20	22	1.0
Quartile 2	6.31–8.07	44	21	2.45 (0.9–7.1)
Quartile 3	8.08–10.54	60	16	3.81 (1.3–11.2)
Quartile 4	>10.54	59	20	1.93 (0.6–5.9)
				$p_{trend} = 0.42$

[a] Includes all women with invasive cervical cancer, regardless of HPV status.
[b] Adjusted for age, ethnicity, study site, number of sexual partners, age at first intercourse, years since last Pap smear, number of pregnancies, smoking status and intensity, oral contraceptive use, education, and income.

in the interview, were more often white, came preferentially from certain study sites, and were of higher socioeconomic status, as measured by education and income. Thus, we explored whether there were differences in participation between the cases and controls that might lead to bias. The cases and controls who donated blood were comparable to each other on age, ethnicity, and study site (Table 1); controls had been individually matched to cases on these factors in the original study design. To examine whether cases and controls differentially participated in the blood draw component by socioeconomic status, age at first intercourse, number of sexual partners, time since last Pap smear, vitamin supplement use, or other cervical cancer risk factors, we compared ORs among all the interviewed subjects and among only those participating in the blood draw. For each of these exposures, similar patterns of risk were seen, suggesting that participation bias was minimal.

To examine the possibility of systemic effects of disease, we compared homocysteine levels in the Stage I (n = 109) and II (n = 30) cases, who had been included in the analysis, with those of the Stage III (n = 10) and IV (n = 5) cases, who had been excluded. Adjusted mean homocysteine levels were similar between Stage I and II cases (Table 4a), and were modestly elevated in the Stage III and IV cases. However, none of the means differed significantly.

In addition, we compared mean homocysteine levels of the cases included in the analysis by a combination of treatment received (surgery or radiation) and cancer stage (Table 4b). None of these women had received chemotherapy. Women who received radiation therapy and were either in Stage I or Stage II of disease had modestly (15–20%) higher adjusted mean homocysteine levels than women who had surgery alone and were in Stage I of disease; however, the differences were not statistically significant. (Only one woman who had surgery alone was in Stage II of disease and she is therefore not included.) Furthermore, when analyses were restricted to the Stage I cases who had surgery alone, a clear elevation in risk was still noted in the top

Table 4. Adjusted mean homocysteine values (μmol/L) (a) by stage of cervical cancer, (b) by treatment and stage of cervical cancer

	No.	Study-design adjusted mean[a] (95% CI)	Multivariate-adjusted mean[b] (95% CI)
a) Stage			
Included in analysis			
Stage I	109	9.0 (8.2–9.9)	8.6 (7.1–10.4)
Stage II	30	9.1 (7.7–10.7)	8.7 (6.8–11.1)
Excluded from analysis			
Stage III	10	9.9 (7.5–13.0)	9.2 (6.5–13.1)
Stage IV	5	10.5 (7.2–15.2)	10.9 (7.0–17.0)
b) Treatment, stage			
Surgery only, Stage I	68	8.5 (7.6–9.6)	8.0 (6.5–9.8)
Any radiation, Stage I	35	9.7 (8.3–11.3)	9.2 (7.1–11.8)
Any radiation, Stage II	25	9.3 (7.8–11.2)	10.0 (7.6-13.2)

[a] Adjusted for age, ethnicity, study site.
[b] Also adjusted for number of sexual partners, age at first intercourse, years since last Pap smear, number of pregnancies, smoking status and intensity, smoking in the past month, oral contraceptive use, history of non-specific genital infection, and education.

three homocysteine quartiles; the adjusted ORs for the lowest to highest quartiles were: 1.0, 2.1, 2.0, and 1.9 (p for trend = 0.27).

Discussion

Serum homocysteine levels were strongly and significantly related to risk of invasive cervical cancer. Compared with women in the lowest homocysteine quartile, women with higher homocysteine levels (≥6.31 μmol/L) had 2–3 times the risk of invasive cervical cancer. This association could reflect an underlying inadequacy of folate, B_{12}, and/or B_6, a genetic polymorphism in MTHFR, or genetic polymorphisms in other critical enzymes.

These risks are much more pronounced than those previously reported for folate. A modest protective effect of folate has been suggested in some, but not all, studies of non-invasive cervical abnormalities [3, 8–14]. We found in the current study population [5] that folate intake was not related to risk of invasive cervical cancer [5] (adjusted OR = 0.85, 95% CI 0.5–1.5, for lowest, relative to highest, quartile), and three other studies of invasive cancer also reported no association with dietary [4, 6] or serum [7] folate status.

Homocysteine may be more predictive of cervical cancer risk than low folate simply because of problems in assessing dietary [40] and blood folate status [41]. However, we believe homocysteine is identifying pervasive abnormalities in one-carbon metabolism. In addition to indicating folate inadequacy, homocysteine can be elevated in response to low B_{12} because this micronutrient, like folate, is necessary for the conversion of homocysteine to methionine, or in response to low B_6, which is needed for homocysteine degradation [19, 29–30]. Genetic polymorphisms that modulate enzyme activity in the one-carbon metabolism pathway, such as MTHFR ($^{667}C \rightarrow T$), can also result in elevated homocysteine levels [18]. Homocysteine is an emerging biomarker for inefficient one-carbon metabolism, which can lead to problems in DNA synthesis, repair, and methylation, any of which can cause cancer [21].

HPV infection is believed to cause nearly all cases of cervical cancer, although only a small minority of women who are HPV-positive progress to cervical cancer [42]. Since in our study cervical cancer risk remained elevated 2–3-fold among women with higher homocysteine levels when analyses were restricted to the HPV-16 seropositive controls, inefficient one-carbon metabolism may be involved in the progression of cervical neoplasia following HPV infection. One plausible mechanism is that HPV may integrate into the host genome at or near chromosomal fragile sites caused by folate inadequacy [22, 25–27]. Among controls, elevated homocysteine levels were not indicative of HPV-16 seropositivity, suggesting that inefficient one-carbon metabolism may not be important in HPV infection, although we cannot rule out this possibility.

Our serologic characterization of HPV infection did have several important limitations. The HPV-16 virus-like particle ELISA test, which uses serum, may be insensitive relative to DNA hybridization assays, which require cervical tissue scrapings [42], and HPV antibody titers may decrease after surgical treatment for cervical cancer [43]. Furthermore, we tested only for antibodies to HPV-16, the most prevalent oncogenic HPV type, which accounts for more than 50% of invasive cervical cancer in the US [23]. A mix of other HPV types accounts for most other cervical cancers. However, it was reassuring that 15% of our controls tested seropositive for HPV-16, similar to a 12% prevalence recently reported among US blood donors [36].

We found no evidence that uncontrolled confounding by associated risk factors accounted for the increased cervical cancer risk associated with high homocysteine levels. Addition of HPV-16 serologic status to the multivariate model adjusted for all other risk factors actually increased the risk for homocysteine, so it is unlikely that better measurement of history of HPV infection would substantially attenuate the effect. In addition, we controlled for age at first intercourse and number of sexual partners to minimize uncontrolled confounding by history of HPV infection. Furthermore, inclusion in the models of education and income, indicators of socioeconomic status, only slightly attenuated the homocysteine effect. Elevated homocysteine did not seem to be a non-specific indicator of poor diet or unhealthy lifestyle as adjustment for dietary vitamin C and carotenoids did not attenuate the homocysteine association.

Participation bias is unlikely to explain our findings. Cases and controls did not differ from each other on the matching factors of the original study design (age, ethnicity, and study site). Subjects who participated in the blood draw did differ from subjects who did not, on education and income, but differential participation was similar for cases and controls. The same patterns of risk were seen for education, income, and other cervical cancer risk factors in all subjects interviewed and in the subgroup who participated in the blood draw.

Neither disease progression nor treatment readily explains our results. Women with Stage I and II tumors, comprising 93% of the cases, had comparable homocysteine levels. To be conservative, we excluded cases with Stage III or IV tumors from analyses, even though their

homocysteine levels were not significantly different from the women with earlier-stage tumors. To minimize the influence of cancer treatment, blood was collected at least 6 months after completion of any treatment. In addition, we excluded the 4% of women who received chemotherapy. When analyses were restricted to Stage I cases who had surgery alone (*i.e.* the subjects with the least advanced disease stage who had not had radiation or chemotherapy), risk remained clearly elevated among women in the three highest homocysteine quartiles.

It is unlikely that the homocysteine would have degraded during storage. Among controls in our study, the 5th–95th percentile range of homocysteine was 4.5–15.1 μmol/L, similar to that reported in the Third National Health and Nutrition Examination Survey [39]. In addition, Israelsson *et al.* [44] found that homocysteine was stable in plasma samples frozen for up to 10 years at −20 °C.

Prospective studies reduce the possibility that disease or treatment may influence results. Therefore, it is reassuring that in a small case–control study nested in the Washington County, MD, cohort, which included 26 *in-situ* and 13 invasive cervical cancer cases, higher homocysteine levels were associated with increased risk of cervical disease (RR = 2.7 for highest tertile of serum homocysteine relative to lowest, for p trend = 0.05) [45]. However, due to the low incidence of invasive cervical cancer in the US, cohort studies are unlikely to yield sufficient numbers of invasive cases for robust analyses of all stages of cervical carcinogenesis, and well-designed retrospective studies will continue to contribute to research in this area.

Very few studies have examined homocysteine and cancer risk. Two studies reported direct relationships between homocysteine and colorectal cancer risk, similar to our finding and to the cervical cancer study described above. One of these studies found a progressive increase in risk with increasing homocysteine levels (OR for highest relative to lowest quartile = 1.72, 95% CI = 0.83–3.7, p for trend = 0.09) [46] and the other found a decreased risk among subjects with the MTHFR C677T polymorphism and homocysteine levels in the lowest two tertiles (OR = 0.25, 95% CI = 0.08–0.74) [47] However, a study of pancreatic cancer found that high homocysteine levels were inversely (but non-significantly) associated with risk (OR for highest relative to lowest tertile = 0.65, 95% CI = 0.36–1.2, p for trend = 0.14) [48], and another study reported a direct, non-significant association between homocysteine and one type of cervical dysplasia, but a negative, non-significant association with another type of cervical dysplasia [14].

In summary, high serum homocysteine levels (> 6.31 μmol/L) were strongly predictive of increased invasive cervical cancer risk in a large, multicenter community-based case–control study in the US, which included serologic measurement of HPV-16 exposure and careful assessment of other cervical cancer risk factors. National surveys have reported serum homocysteine above this level in a substantial fraction of US women. While this association may reflect a critical role for folate in cervical carcinogenesis, it may also be related to inadequate levels of vitamins B_{12} or B_6, or variability in one-carbon metabolism genes. Homocysteine may be an informative biomarker to incorporate into studies of other cancers in which folate inadequacies have been suggested, such as colon, lung, esophagus, stomach, and brain [49, 50]. Homocysteine can serve as an integratory biomarker of inefficiencies in one-carbon metabolism, and may help elucidate the role of these problems in human carcinogenesis.

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